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DATE: Tuesday, August 17, 2004

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*DB=USPT; PLUR=YES; OP=AND*

☐ L1 (portner or ginnny).xa.

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*DB=USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=OR*

☐ L3 ((neospora or neosporosis or neospor\$) not neosporin\$ near5 (avirulent or a-virulent or non-virulent or attenuated or attenuation or attenuating or modified or modification of mutagenesis or mutation or mutant or alter or alteration or altered or altering))

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- 
- ☐ 1. [6777192](#). 21 Sep 01; 17 Aug 04. Recombinant neospora antigens and their uses. Conrad; Patricia A., et al. 435/7.1; 435/7.21 435/7.92. G01N033/53 G01N033/567 G01N033/537.
- 
- ☐ 2. [6716423](#). 10 Jul 00; 06 Apr 04. Recombinant neospora antigens and their uses. Conrad; Patricia A., et al. 424/93.1; 424/184.1 424/234.1 424/93.7. A61K039/00 A61K039/38 A61K039/02 A01N063/00 A01N065/00.
- 
- ☐ 3. [6656479](#). 12 Sep 01; 02 Dec 03. Attenuated live neospora vaccine. Brake; David A, et al. 424/269.1; 424/258.1 424/271.1 424/273.1 424/93.1 424/93.2 435/258.1 435/69.1. A61K039/002.
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- ☐ 4. [6600027](#). 25 Mar 99; 29 Jul 03. Polynucleotide molecules encoding neospora proteins. Krishnan; B. Rajendra, et al. 536/23.1; 435/252.3 435/320.1 435/69.1 536/23.4 536/23.5 536/24.32. C07H021/02 C07H021/04 C12P021/06 C12N001/20 C12N015/00.
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- ☐ 5. [6436410](#). 02 Dec 98; 20 Aug 02. DNA encoding neospora dihydrofolate reductase-thymidylate synthase. Krishnan; B. Rajendra, et al. 424/265.1; 424/184.1 424/191.1 424/200.1 424/273.1 435/6 435/91.4 536/23.1 536/23.7. A61K039/002 A61K039/012 C07H021/04 C07H021/02 C12Q001/68.
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- ☐ 6. [6376196](#). 30 Mar 99; 23 Apr 02. Recombinant neospora antigens and their uses. Conrad; Patricia, et al. 435/7.1; 435/7.21 435/7.92. G01N033/53 G01N033/567 G01N033/537.
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- ☐ 7. [6071737](#). 16 Mar 98; 06 Jun 00. Equine Neospora isolate and its uses. Marsh; Antoinette E., et al. 435/258.1;. C12N001/14.
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- ☐ 8. [5889166](#). 10 May 96; 30 Mar 99. Recombinant neospora antigens and their uses. Conrad; Patricia A., et al. 536/23.1; 530/300 530/350 530/371. C07H021/02 A61K038/00 C07K001/00.
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- ☐ 9. [5707617](#). 20 Oct 94; 13 Jan 98. Bovine neospora isolates. Conrad; Patricia A., et al. 424/93.1; 435/258.1. C12N001/10.
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- ☐ 10. [JP410167983A](#). 12 Nov 97. 23 Jun 98. ATTENUATED LIVE NEOSPORA VACCINE. DAVID, A BRAKE, et al. A61K039/00; A61K035/68 A61K039/002 A61K039/39 C12N001/00.
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- ☐ 11. [EP001221485A2](#). 09 Mar 99. 10 Jul 02. Polynucleotide molecules encoding neospora proteins. BRAKE, DAVID ALAN, et al. C12N015/30; C12N015/85 C12N005/10 C07K014/44 C07K016/20 A61K039/002.
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- ☐ 12. [EP000953641A2](#). 09 Mar 99. 03 Nov 99. Polynucleotide molecules encoding neospora proteins. BRAKE, DAVID ALAN, et al. C12N015/30; C07K014/44 C07K016/20 C12N005/16 A61K039/002.
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- ☐ 13. [EP000924295A2](#). 26 Nov 98. 23 Jun 99. DNA encoding neospora dihydrofolate reductase-thymidylate synthase. KRISHNAN, BALAKRISHNAN RAJENDRA, et al. C12N009/06; C12N015/53 C12N015/73 C07K016/40 C12N001/11 A61K039/002.
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et al. C12N001/10; A61K039/002 A61K039/00.

- ☐ 15. US20030044431A. New overexpressing homologous antigen vaccines, useful for immunization, prophylaxis or treatment of a vertebrate at risk of or suffering from, e.g. brucellosis, paratuberculosis, tuberculosis or neosporosis. BOYLE, S M, et al. A61K039/10.
- ☐ 16. EP 1221485A. Novel *Neospora caninum* GRA2 protein useful for producing vaccines against neosporosis and as diagnostic reagents. BRAKE, D A, et al. A61K039/002 C07K014/44 C07K016/20 C12N005/10 C12N015/30 C12N015/85.
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- ☐ 20. NZ 329095A. Live attenuated *Neospora* vaccine - for protecting animals against neosporosis. BLAGBURN, B, et al. A61K000/00 A61K035/68 A61K039/00 A61K039/002 A61K039/02 A61K039/38 A61K039/39 C12N001/00 C12N001/10 C12N001/11 C12N001/13 C12N001/18 C12N001/36 C12N005/06 C12N015/30 C12N001/10 C12R001:90 C12N001/10 C12R001:90.

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L3 near5 (avirulent or a-virulent or non-virulent or attenuated or attenuation or attenuating or modified or modification of mutagenesis or mutation or mutant or alter or alteration or altered or altering)	20

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L4: Entry 1 of 20

File: USPT

Aug 17, 2004

US-PAT-NO: 6777192

DOCUMENT-IDENTIFIER: US 6777192 B2

TITLE: Recombinant neospora antigens and their uses

DATE-ISSUED: August 17, 2004

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Conrad; Patricia A.	Davis	CA		
Barr; Bradd C.	Davis	CA		
Anderson; Mark L.	Davis	CA		
Sverlow; Karen W.	Vacaville	CA		

## ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE	CODE
The Regents of the University of California	Oakland	CA				02

APPL-NO: 09/ 957995   [PALM]

DATE FILED: September 21, 2001

## PARENT-CASE:

This application is a continuation of U.S. Ser. No. 09/281,766, filed Mar. 30, 1999, now U.S. Pat. No. 6,376,196, which is a continuation-in-part of U.S. Ser. No. 08/645,951, filed May 10, 1996, now U.S. Pat. No. 5,889,166, which is a continuation-in-part of U.S. Ser. No. 08/327,516, filed Oct. 20, 1994, now U.S. Pat. No. 5,707,617, which is a continuation-in-part of U.S. Ser. No. 08/215,858, filed Mar. 21, 1994, now abandoned.

INT-CL: [07] G01 N 33/53, G01 N 33/567, G01 N 33/537

US-CL-ISSUED: 435/7.1; 435/7.21, 435/7.92

US-CL-CURRENT: 435/7.1; 435/7.21, 435/7.92

FIELD-OF-SEARCH: 424/184.1, 435/7.1, 435/7.21, 435/7.92, 530/350

PRIOR-ART-DISCLOSED:

## U.S. PATENT DOCUMENTS

PAT-NO

ISSUE-DATE

PATENTEE-NAME

US-CL

4431739

February 1984

Riggs et al.

<input type="checkbox"/> <u>5707617</u>	January 1998	Conrad et al.
<input type="checkbox"/> <u>6376196</u>	April 2002	Conrad et al.

## FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
WO 95/25541	September 1995	WO	
WO 97/39009	October 1997	WO	

## OTHER PUBLICATIONS

Levinson et al (Medical Microbiology & Immunology p. 263), 1989.\*  
Dubey et al (Journal of Parasitology vol. 75(5) pp 765-771), 1989.\*  
Lindsay et al (J. of Helminthological Society of Washington vol. 57(1) pp 86-88), 1990.\*  
Barr et al., Congenital Neospora infection in calves born from cows that had previously aborted Neospora-infected fetuses: Four cases (1990-1992), JAVMA 202:1: 113-117 (1993).  
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ART-UNIT: 1645

PRIMARY-EXAMINER: Navarro; Mark

ATTY-AGENT-FIRM: Townsend and Townsend and Crew LLP


## ABSTRACT:

The present invention provides isolated bovine Neospora cultures. Also provided are recombinant immunodominant Neospora antigens. The cultures and antigens are used to develop diagnostic assays for the detection of Neospora infections in cattle and other animals. Also provided are pharmaceutical compositions for the treatment and prevention of Neospora infections.

5 Claims, 6 Drawing figures

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# Search in Swiss-Prot and TrEMBL for: neospora

**Swiss-Prot Release 44.3 of 16-Aug-2004**

**TrEMBL Release 27.3 of 16-Aug-2004**

- 
- Number of sequences found in [Swiss-Prot](#)<sub>(3)</sub> and [TrEMBL](#)<sub>(39)</sub>: **42**
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- 

## Search in Swiss-Prot: There are matches to 3 out of 156998 entries

[1433\\_NEOCA \(Q25538\)](#)

14-3-3 protein homolog. - Neospora caninum

[GRA1\\_NEOCA \(P90661\)](#)

Dense granule protein 1 precursor (NcDG1) (Antigen Nc4.1). {GENE: Name=DG1} - Neospora caninum

[GRA2\\_NEOCA \(Q25540\)](#)

Dense granule protein 2 (NcDG2) (Antigen Nc14.1). {GENE: Name=DG2} - Neospora caninum

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## Search in TrEMBL: There are matches to 39 out of 1379120 entries

[O44063](#)

Antigen N54 (Fragment) - Neospora sp

[O61159](#)

Surface protein Nc-p43 - Neospora caninum

[O62617](#)

P36 protein precursor (Surface protein P36) {GENE:Name=p36} - Neospora caninum

[O76144](#)

NTPase - Neospora caninum

[O96451](#)

Surface antigen p35 - Neospora caninum

[Q6EEV5](#)

Apical complex protein (Fragment) - Neospora caninum

[Q6IMH5](#)

Rbj-like protein (Fragment) {GENE:Name=Rjl} - Neospora caninum

Q6YDA6

Putative dense granule protein 3 - Neospora caninum

Q6YFA5

Small heat shock protein - Neospora caninum

Q6YFA6

Small heat shock protein (Fragment) - Neospora caninum

Q71G51

Alpha-tubulin {GENE:Name=TUBA} - Neospora caninum

Q75UX7

Chitinase 19-1 (Fragment) {GENE:Name=chi19-1} - Actinokineospora riparia

Q86M53

Putative surface antigen protein 1 {GENE:Name=SAG1} - Neospora caninum

Q8IT72

Microneme protein NcMIC11 precursor {GENE:Name=MIC11} - Neospora caninum

Q8MZJ9

Serine proteinase inhibitor PI-S - Neospora caninum

Q8WRS0

Microneme-associated protein {GENE:Name=MIC1} - Neospora caninum

Q8WSI9

Superoxide dismutase {GENE:Name=SOD} - Neospora caninum

Q962A6

MIC2-associated protein precursor {GENE:Name=M2AP} - Neospora caninum

Q9BKL9

P20 - Neospora caninum

Q9BKM0

Gra1 (Fragment) {GENE:Name=GRA1} - Neospora caninum

Q9GSV4

NcMIC10 precursor {GENE:Name=MIC10} - Neospora caninum

Q9GTB7

Myosin E (Fragment) - Neospora caninum

Q9GTB8

Myosin D (Fragment) - Neospora caninum

Q9GTB9

Myosin C (Fragment) - Neospora caninum

Q9GTC0

Myosin B (Fragment) - Neospora caninum

Q9GTC1

Myosin A (Fragment) - Neospora caninum

Q9GU48

GRA2 protein {GENE:Name=GRA2} - Neospora caninum

Q9NB95

SUL1 - Neospora caninum

Q9NJJ4

DNA dependent RNA polymerase beta subunit' (Fragment) {GENE:Name=rpoC1} - Neospora caninum

Q9NJJ5

DNA dependent RNA polymerase beta subunit {GENE:Name=rpoB} - Neospora caninum

Q9NJS9

SAG1 precursor {GENE:Name=SAG1} - Neospora hughesi

Q9TVP5

SRS2 surface antigen (Fragment) {GENE:Name=srs2} - Neospora caninum



[Q9TVV8](#)

P29 surface antigen (Fragment) {GENE:Name=SAG1} - Neospora caninum

[Q9U483](#)

Microneme protein Nc-P38 - Neospora caninum

[Q9U4R7](#)

SAG1-related sequence 2 {GENE:Name=SRS2} - Neospora hughesi

[Q9U7D4](#)

Subtilisin-like serine protease (Fragment) - Neospora caninum

[Q9U7D6](#)

Ycf24 protein (Fragment) {GENE:Name=ycf24} - Neospora caninum

[Q9U8J9](#)

Thrombospondin-related adhesive protein homolog {GENE:Name=MIC2} - Neospora caninum

[Q9UB12](#)

Surface antigen SAG1 {GENE:Name=SAG1} - Neospora caninum

in Swiss-Prot/TrEMBL by AC, ID, description,  
gene name, organism


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## Entry information

Entry name	<b>1433_NEOCA</b>
Primary accession number	<b>Q25538</b>
Secondary accession numbers	None
Entered in Swiss-Prot in	Release 36, July 1998
Sequence was last modified in	Release 36, July 1998
Annotations were last modified in	Release 36, July 1998
<b>Name and origin of the protein</b>	
Protein name	<b>14-3-3 protein homolog</b>
Synonyms	None
Gene name	None
From	<u>Neospora caninum</u> [TaxID: 29176]
Taxonomy	<u>Eukaryota</u> ; <u>Alveolata</u> ; <u>Apicomplexa</u> ; <u>Coccidia</u> ; <u>Eimeriida</u> ; <u>Sarcocystidae</u> ; <u>Neospora</u> .

## References

[1] SEQUENCE FROM NUCLEIC ACID.  
**STRAIN**=Nc-1;  
 MEDLINE=96258557;PubMed=8992315 [NCBI, ExPASy, EBI, Israel, Japan]  
Lally N.C., Jenkins M.C., Dubey J.P.;  
 "Development of a polymerase chain reaction assay for the diagnosis of neosporosis using the  
 Neospora caninum 14-3-3 gene."  
 Mol. Biochem. Parasitol. 75:169-178(1996).


## Comments

- **SIMILARITY**: Belongs to the 14-3-3 family.

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### Entry information

Entry name	<b>GRA1_NEOCA</b>
Primary accession number	<b>P90661</b>
Secondary accession numbers	O02507 Q25539
Entered in Swiss-Prot in	Release 36, July 1998
Sequence was last modified in	Release 36, July 1998
Annotations were last modified in	Release 44, July 2004
<b>Name and origin of the protein</b>	
Protein name	<b>Dense granule protein 1 [Precursor]</b>
Synonyms	<b>NcDG1</b> <b>Antigen Nc4.1</b>
Gene name	<b>Name: DG1</b>
From	<u>Neospora caninum</u> [TaxID: 29176]
Taxonomy	<u>Eukaryota</u> ; <u>Alveolata</u> ; <u>Apicomplexa</u> ; <u>Coccidia</u> ; <u>Eimeriida</u> ; <u>Sarcocystidae</u> ; <u>Neospora</u> .

### References

- [1] SEQUENCE FROM NUCLEIC ACID.  
**STRAIN=Nc-1**;  
 MEDLINE=97391130;PubMed=9247937 [[NCBI](#), [ExPASy](#), [EBI](#), [Israel](#), [Japan](#)]  
[Lally N.C.](#), [Jenkins M.C.](#), [Liddell S.](#), [Dubey J.P.](#);  
 "A dense granule protein (NCDG1) gene from *Neospora caninum*."  
 Mol. Biochem. Parasitol. 87:239-243(1997).
- [2] SEQUENCE OF 15-217 FROM NUCLEIC ACID.  
**STRAIN=Nc-1**;  
 MEDLINE=96336081;PubMed=8705668 [[NCBI](#), [ExPASy](#), [EBI](#), [Israel](#), [Japan](#)]  
[Lally N.C.](#), [Jenkins M.C.](#), [Dubey J.P.](#);  
 "Evaluation of two *Neospora caninum* recombinant antigens for use in an enzyme-linked immunosorbent assay for the diagnosis of bovine neosporosis."  
 Clin. Diagn. Lab. Immunol. 3:275-279(1996).

### Comments

- **SUBCELLULAR LOCATION:** Secreted (*Potential*).
- **SIMILARITY:** Belongs to the Gra7 family.

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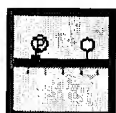
### Cross-references

EMBL	U72991; AAB48402.1; -. [EMBL / GenBank / DDBJ] [CoDingSequence] U82229; AAC47661.1; -. [EMBL / GenBank / DDBJ] [CoDingSequence] U36386; AAC47096.1; -. [EMBL / GenBank / DDBJ] [CoDingSequence]
InterPro	IPR008120; Gra7_protein. <a href="#">Graphical view of domain structure.</a>
PRINTS	PR01747; DENSEGRNULE7.
ProDom	[ <a href="#">Domain structure</a> / <a href="#">List of seq. sharing at least 1 domain</a> ]
BLOCKS	P90661.
ProtoNet	P90661.
ProtoMap	P90661.
PRESAGE	P90661.
DIP	P90661.
ModBase	P90661.
SMR	P90661; 098B4C1B33705CC2.
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### Keywords

**Antigen; Signal.**

### Features



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Key	From	To	Length	Description
SIGNAL	<u>1</u>	<u>19</u>	19	<i>Potential.</i>
CHAIN	<u>20</u>	<u>217</u>	198	Dense granule protein 1.
CARBOHYD	<u>191</u>	<u>191</u>		N-linked (GlcNAc...) ( <i>Potential</i> ).
CONFLICT	<u>15</u>	<u>18</u>		GLAI -> IRHE (in Ref. <u>2</u> ).

### Sequence information

Length: **217 AA** [This is the length of the unprocessed precursor]

Molecular weight: **22495 Da** [This is the MW of the unprocessed precursor]

CRC64: **098B4C1B33705CC2** [This is a checksum on the sequence]

10	20	30	40	50	60
MARQATFIVA	LCVCGLAIAG	LPRLASAGDL	ATEQHEGDIG	YGVRAYAGVS	NYDGDDDAAG
70	80	90	100	110	120
NPVDSDVTD	AITDGEWPRV	VSGQKPHTTQ	KGSLIKKLAV	PVVGALTSYL	VADRVLPFLT
130	140	150	160	170	180

```
SAEEEGTESI PGKKRVKTAV GIAALVAAAA FAGLGLARTF RHFVPKKSKT VASEDSALGN
      190      200      210
SEEQYVEGTV NGSSDPEQER AGGPLIPEGD EQEVDTE
```

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## Q25540

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[\[Sequence\]](#)
[\[Tools\]](#)

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### Entry information

Entry name	<b>GRA2_NEOCA</b>
Primary accession number	<b>Q25540</b>
Secondary accession number	O76215
Entered in Swiss-Prot in	Release 36, July 1998
Sequence was last modified in	Release 42, October 2003
Annotations were last modified in	Release 44, July 2004
<b>Name and origin of the protein</b>	
Protein name	<b>Dense granule protein 2</b>
Synonyms	<b>NcDG2</b> <b>Antigen Nc14.1</b>
Gene name	<b>Name: DG2</b>
From	<a href="#">Neospora caninum</a> [TaxID: <a href="#">29176</a> ]
Taxonomy	<a href="#">Eukaryota</a> ; <a href="#">Alveolata</a> ; <a href="#">Apicomplexa</a> ; <a href="#">Coccidia</a> ; <a href="#">Eimeriida</a> ; <a href="#">Sarcocystidae</a> ; <a href="#">Neospora</a> .

### References

#### [1] SEQUENCE FROM NUCLEIC ACID.

**STRAIN**=Nc-1;

MEDLINE=98324416;PubMed=9662039 [[NCBI](#), [ExPASy](#), [EBI](#), [Israel](#), [Japan](#)]

[Liddell S.](#), [Lally N.C.](#), [Jenkins M.C.](#), [Dubey J.P.](#);

"Isolation of the cDNA encoding a dense granule associated antigen (NCDG2) of *Neospora caninum*.";

Mol. Biochem. Parasitol. 93:153-158(1998).

#### [2] SEQUENCE OF 67-193 FROM NUCLEIC ACID.

**STRAIN**=Nc-1;

MEDLINE=96336081;PubMed=8705668 [[NCBI](#), [ExPASy](#), [EBI](#), [Israel](#), [Japan](#)]

[Lally N.C.](#), [Jenkins M.C.](#), [Dubey J.P.](#);

"Evaluation of two *Neospora caninum* recombinant antigens for use in an enzyme-linked immunosorbent assay for the diagnosis of bovine neosporosis.";

Clin. Diagn. Lab. Immunol. 3:275-279(1996).

**Comments**

- **SIMILARITY:** Belongs to the Gra6 family.

**Copyright**

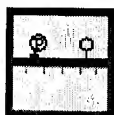
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**Cross-references**

EMBL	AF029350; AAC39122.1; -. [ <a href="#">EMBL</a> / <a href="#">GenBank</a> / <a href="#">DDBJ</a> ] [ <a href="#">CoDingSequence</a> ] U36387; AAC47097.1; -. [ <a href="#">EMBL</a> / <a href="#">GenBank</a> / <a href="#">DDBJ</a> ] [ <a href="#">CoDingSequence</a> ]
InterPro	<a href="#">IPR008119</a> ; Gra6_protein. <a href="#">Graphical view of domain structure.</a>
Pfam	<a href="#">PF05084</a> ; GRA6; 1. <a href="#">Pfam graphical view of domain structure.</a>
ProDom	<a href="#">[Domain structure / List of seq. sharing at least 1 domain]</a>
BLOCKS	<a href="#">Q25540.</a>
ProtoNet	<a href="#">Q25540.</a>
ProtoMap	<a href="#">Q25540.</a>
PRESAGE	<a href="#">Q25540.</a>
DIP	<a href="#">Q25540.</a>
ModBase	<a href="#">Q25540.</a>
SMR	<a href="#">Q25540</a> ; 7EF46F94C289BC0B.
SWISS-2DPAGE	<a href="#">Get region on 2D PAGE.</a>
UniRef	View cluster of proteins with at least <u>50%</u> / <u>90%</u> identity.

**Keywords**

**Antigen**; **Transmembrane**.

**Features**

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[Feature aligner](#)

Key	From	To	Length	Description
TRANSMEM	<a href="#">14</a>	<a href="#">34</a>	21	Potential.
TRANSMEM	<a href="#">153</a>	<a href="#">173</a>	21	Potential.
CARBOHYD	<a href="#">4</a>	<a href="#">4</a>		N-linked (GlcNAc...) (Potential).
CARBOHYD	<a href="#">74</a>	<a href="#">74</a>		N-linked (GlcNAc...) (Potential).
CONFLICT	<a href="#">67</a>	<a href="#">70</a>		GTSE -> NSAR (in Ref. <a href="#">2</a> ).

**Sequence information**

Length: **193** Molecular weight: **20590** CRC64: **7EF46F94C289BC0B** [This is a checksum on the AA Da sequence]

10	20	30	40	50	60
MANNRTLARR	RRAFSPLTVV	MLAVTLVAFM	GVPLSSTGAA	DAADPVESVE	ANRRGYTSYG
70	80	90	100	110	120
EPPVAVGTSE	EYVNSSELAG	SRDKGNAEAE	EEAAEVETDV	QPSSVTIDTE	ERAAPSQVQV
130	140	150	160	170	180

Q Q E R M E E A D D   A P K P V P V R S A   V P S T V A K R Q Q   A R H R V I G T A V   I A A V V A A L L W   K F S R R R S G A P

190

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## O61159

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### Entry information

Entry name	<b>O61159</b>
Primary accession number	<b>O61159</b>
Secondary accession numbers	None
Entered in TrEMBL in	Release 07, August 1998
Sequence was last modified in	Release 07, August 1998
Annotations were last modified in	Release 26, March 2004
<b>Name and origin of the protein</b>	
Protein name	<b>Surface protein Nc-p43</b>
Synonyms	None
Gene name	None
From	<u>Neospora caninum</u> [TaxID: 29176]
Taxonomy	<u>Eukaryota</u> ; <u>Alveolata</u> ; <u>Apicomplexa</u> ; <u>Coccidia</u> ; <u>Eimeriida</u> ; <u>Sarcocystidae</u> ; <u>Neospora</u> .

### References

- [1] SEQUENCE FROM NUCLEIC ACID.  
**STRAIN**=Nc-1;  
 MEDLINE=96429628;PubMed=8832729 [[NCBI](#), [ExPASy](#), [EBI](#), [Israel](#), [Japan](#)]  
[Hemphill A.](#), [Gottstein B.](#);  
 "Identification of a major surface protein on Neospora caninum tachyzoites.";  
 Parasitol. Res. 82:497-504(1996).
- [2] SEQUENCE FROM NUCLEIC ACID.  
**STRAIN**=Nc-1;  
 MEDLINE=97047711;PubMed=8926100 [[NCBI](#), [ExPASy](#), [EBI](#), [Israel](#), [Japan](#)]  
[Hemphill A.](#);  
 "Subcellular localization and functional characterization of Nc-p43, a major Neospora caninum tachyzoite surface protein.";  
 Infect. Immun. 64:4279-4287(1996).
- [3] SEQUENCE FROM NUCLEIC ACID.  
**STRAIN**=Nc-1;  
 MEDLINE=98150127;PubMed=9488869 [[NCBI](#), [ExPASy](#), [EBI](#), [Israel](#), [Japan](#)]  
[Hemphill A.](#), [Felleisen R.](#), [Connolly B.](#), [Gottstein B.](#), [Hentrich B.](#), [Muller N.](#);

"Characterization of a cDNA-clone encoding Nc-p43, a major Neospora caninum tachyzoite surface protein.";

Parasitology 115:581-590(1997).

[4] SEQUENCE FROM NUCLEIC ACID.

**STRAIN**=Nc-1;

Hehl A., Felleisen R., Sonda S., Fuchs N., Muller N., Hemphill A.;

Submitted (APR-1998) to the EMBL/GenBank/DDBJ databases.

**Comments**

None

**Cross-references**

EMBL U93870; AAC15250.1; -.[\[EMBL / GenBank / DDBJ\]](#) [\[CoDingSequence\]](#)

HSSP P13664; 1KZQ. [\[HSSP ENTRY / PDB\]](#)

InterPro [IPR007226](#); SAG.  
[Graphical view of domain structure.](#)

Pfam [PF04092](#); SAG; 2.  
[Pfam graphical view of domain structure.](#)

PRINTS [PR01801](#); SURFCEANTIGN.

ProDom [\[Domain structure / List of seq. sharing at least 1 domain\]](#)

ProtoMap [O61159](#).

PRESAGE [O61159](#).

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SMR [O61159](#); CA52F48B3E7DD360.

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UniRef [View cluster of proteins with at least 50% / 90% identity.](#)

**Keywords**

None

**Features**

None

**Sequence information**

Length: **400** Molecular weight: **42051** CRC64: **CA52F48B3E7DD360** [This is a checksum on the  
**AA** **Da** sequence]

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MATHACVRR	KADAACFAKL	SASQSCLTAK	SVNRSVSVFA	LLFGVVLAVG	VSGAPFKSEN
70	80	90	100	110	120
EKFTCLPKQG	NADQWVALVY	DSQHSITFAC	DGGRPPSKLL	SEDDGLIVCN	ESDGEDECEK
130	140	150	160	170	180
NAAPLSTFLP	GAKKEWVTGT	LQQGIKITIP	DEHYPATSKA	FRVGCKAGKN	VCLLNIVYVQS
190	200	210	220	230	240
RESEVIGQVA	HCAYSNNVRL	RPITVNPENN	GVTLICGPDG	KAFPDDYMNH	HCTELDECKE
250	260	270	280	290	300
RPYSAVFPGF	SSSFWTGEAS	GVAGATLTIP	KDQFPSTAQT	IYLGCTGHPD	DKQVTCVVPV

```
      310      320      330      340      350      360
      |       |       |       |       |       |
NIEEVAKPAG AGSNPGGGSQ PDQSSEKRDG EQVNKGKPPT GSGRGTTTGK LNASLNAKDK

      370      380      390      400
      |       |       |       |
GETGGENGDS PVLRGDACDE LPSYVALSAA SLTATAIFAY
```

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 \$8.46 Estimated cost File349  
       \$0.15 0.032 DialUnits File348  
       \$1.70 1 Type(s) in Format 3  
       \$1.70 1 Types  
 \$1.85 Estimated cost File348  
       \$0.02 0.006 DialUnits File10  
 \$0.02 Estimated cost File10  
       \$0.13 0.006 DialUnits File357  
       \$6.81 3 Type(s) in Format 3  
       \$6.81 3 Types  
 \$6.94 Estimated cost File357  
       \$0.02 0.006 DialUnits File144  
 \$0.02 Estimated cost File144  
       \$0.02 0.006 DialUnits File203  
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       \$0.02 0.006 DialUnits File358  
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 \$0.07 Estimated cost File347  
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       \$0.02 0.006 DialUnits File65  
 \$0.02 Estimated cost File65  
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 \$0.04 Estimated cost File185  
       \$0.03 0.006 DialUnits File16  
 \$0.03 Estimated cost File16  
       OneSearch, 16 files, 0.440 DialUnits FileOS  
 \$0.24 TELNET  
 \$25.70 Estimated cost this search  
 \$25.70 Estimated total session cost 0.440 DialUnits

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Welcome to DIALOG

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Dialog level 04.11.00D

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 File 348:EUROPEAN PATENTS 1978-2004/Aug W02  
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 File 347:JAPIO Nov 1976-2004/Apr(Updated 040802)  
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 File 344:Chinese Patents Abs Aug 1985-2004/May  
 (c) 2004 European Patent Office  
 File 94:JICST-EPlus 1985-2004/Jul W4  
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 File 65:Inside Conferences 1993-2004/Aug W2  
 (c) 2004 BLDSC all rts. reserv.  
 File 185:Zoological Record Online(R) 1978-2004/Jul  
 (c) 2004 BIOSIS  
 \*File 185: File 185 has been reloaded. All accession  
 numbers have changed. See HELP NEWS185.  
 File 16:Gale Group PROMT(R) 1990-2004/Aug 16  
 (c) 2004 The Gale Group  
 \*File 16: Alert feature enhanced for multiple files, duplicate  
 removal, customized scheduling. See HELP ALERT.

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S1	134	NEOSPOR? (3N) (CULTUR? OR REDUC? OR CHANG? OR MODIFIC? OR - ATTEN? OR DECREAS?)
S2	107	RD (unique items)

?t s2/9/95 97 98

2/9/95 (Item 2 from file: 94)  
 DIALOG(R)File 94:JICST-EPlus  
 (c)2004 Japan Science and Tech Corp(JST). All rts. reserv.

02961647 JICST ACCESSION NUMBER: 97A0890408 FILE SEGMENT: PreJICST-E  
**Examination on culture condition for neospora protozoa.**  
 ETO MARIKO (1); YAMANE ITSURO (1); KUNIYASU TAKEHIRO (1); SHIMURA KAMEO  
 (1); HATAYA RYO (1); OUCHI YOSHINAO (2)  
 (1) Minist. of Agric., For. and Fish., Natl. Inst. of Anim. Health; (2)  
 North Dist. Livest. Hyg. Serv. Cent., Ibaraki Prefect. Gov.  
 Nippon Jui Gakkai Koen Yoshishu, 1996, VOL.122nd, PAGE.119  
 JOURNAL NUMBER: Z0670AAA  
 LANGUAGE: Japanese COUNTRY OF PUBLICATION: Japan  
 DOCUMENT TYPE: Journal  
 MEDIA TYPE: Printed Publication

2/9/97 (Item 1 from file: 65)  
 DIALOG(R)File 65:Inside Conferences  
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02254714    INSIDE CONFERENCE ITEM ID: CN023606104

**Viability determination of culture -grown Neospora caninum**

Jakubek, E.-B.; Uggla, A.; Bjoerkman, C.

CONFERENCE: Control of coccidiosis into the next millennium-International coccidiosis conference; 7th

P: 129

Newbury, Institute for Animal Health, 1997

LANGUAGE: English    DOCUMENT TYPE: Conference Abstracts

CONFERENCE EDITOR(S): Shirley, M. W.; Tomley, F. M.; Freeman, B. M.

CONFERENCE SPONSOR: COST 820    Project

CONFERENCE LOCATION: Oxford

CONFERENCE DATE:    Sep 1997 (199709) (199709)

BRITISH LIBRARY ITEM LOCATION: 98/07811

NOTE:

Held in conjunction with the European Union COST 820 workshop

DESCRIPTORS: coccidiosis; COST

**2/9/98            (Item 2 from file: 65)**

DIALOG(R)File    65:Inside Conferences

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02008929    INSIDE CONFERENCE ITEM ID: CN020883541

**Evaluation of In vivo Inoculations of Culture -Derived Neospora sp. and Sarcocystis neurona Parasites for the Development of the Cyst Stage**

Marsh, A. E.; Barr, B. C.; Tell, L.; Sverlow, K. W.

CONFERENCE: American Association of Veterinary Pathologists-Annual meeting; 41st

PROCEEDINGS-AMERICAN ASSOCIATION OF VETERINARY PATHOLOGISTS, 1996; CONF 41    P: 6

The Association, 1996

LANGUAGE: English    DOCUMENT TYPE: Conference Abstracts and programme

CONFERENCE SPONSOR: American Association of Veterinary Pathologists

CONFERENCE LOCATION: Louisville, KY

CONFERENCE DATE:    Jul 1996 (199607) (199607)

BRITISH LIBRARY ITEM LOCATION: 6622.350000

DESCRIPTORS: AAVP; veterinary pathologists

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\$0.04	Estimated cost	File155	
\$0.07	0.012	DialUnits	File5
\$0.07	Estimated cost	File5	
\$0.07	0.012	DialUnits	File654
\$0.07	Estimated cost	File654	
\$0.06	0.012	DialUnits	File349
\$0.06	Estimated cost	File349	
\$0.06	0.012	DialUnits	File348
\$0.06	Estimated cost	File348	
\$0.03	0.012	DialUnits	File10
\$0.03	Estimated cost	File10	
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\$0.24	Estimated cost	File357	
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\$0.13	0.012	DialUnits	File344
\$0.13	Estimated cost	File344	
\$0.04	0.012	DialUnits	File94

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    \$1.35 1 Types  
\$1.39 Estimated cost File94  
    \$0.14 0.037 DialUnits File65  
    \$2.20 2 Type(s) in Format 9  
    \$2.20 2 Types  
\$2.34 Estimated cost File65  
    \$0.08 0.012 DialUnits File185  
\$0.08 Estimated cost File185  
    \$0.07 0.012 DialUnits File16  
\$0.07 Estimated cost File16  
    OneSearch, 16 files, 0.224 DialUnits FileOS  
\$0.24 TELNET  
\$5.08 Estimated cost this search  
\$5.08 Estimated total session cost 0.224 DialUnits

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\*File 654: Reassignment data now updated monthly.

File 349:PCT FULLTEXT 1979-2002/UB=20040812,UT=20040805

(c) 2004 WIPO/Univentio

File 348:EUROPEAN PATENTS 1978-2004/Aug W02

(c) 2004 European Patent Office

File 10:AGRICOLA 70-2004/Jun

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File 357:Derwent Biotech Res. 1982-2004/Aug W3

(c) 2004 Thomson Derwent & ISI

File 144:Pascal 1973-2004/Aug W2

(c) 2004 INIST/CNRS

File 203:AGRIS 1974-2004/Jun

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File 358:Current BioTech Abs 1983-2004/Jul

(c) 2004 DECHEMA

File 347:JAPIO Nov 1976-2004/Apr(Updated 040802)

(c) 2004 JPO & JAPIO

\*File 347: JAPIO data problems with year 2000 records are now fixed. Alerts have been run. See HELP NEWS 347 for details.

File 344:Chinese Patents Abs Aug 1985-2004/May

(c) 2004 European Patent Office

File 94:JICST-EPlus 1985-2004/Jul W4

(c)2004 Japan Science and Tech Corp(JST)

File 65:Inside Conferences 1993-2004/Aug W2

(c) 2004 BLDSC all rts. reserv.

File 185:Zoological Record Online(R) 1978-2004/Jul

(c) 2004 BIOSIS

\*File 185: File 185 has been reloaded. All accession numbers have changed. See HELP NEWS185.

File 16:Gale Group PROMT(R) 1990-2004/Aug 16

(c) 2004 The Gale Group

\*File 16: Alert feature enhanced for multiple files, duplicate removal, customized scheduling. See HELP ALERT.

Set Items Description

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Cost is in DialUnits

?ds

Set Items Description

S1 134 NEOSPOR? (3N) (CULTUR? OR REDUC? OR CHANG? OR MODIFIC? OR -  
ATTEN? OR DECREAS?)

S2 107 RD (unique items)

?t s2/9/9

2/9/9 (Item 9 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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10923841 PMID: 11068819

**Humoral immune responses and safety of experimental formulations of inactivated Neospora vaccines.**

Choromanski L; Block W

Bayer, Agriculture Division, Animal Health, Shawnee Mission, KS 66201, USA.

Parasitology research (GERMANY) Oct 2000, 86 (10) p851-3, ISSN 0932-0113 Journal Code: 8703571

Document type: Journal Article



Languages: ENGLISH  
Main Citation Owner: NLM  
Record type: Completed  
Subfile: INDEX MEDICUS

Antibody titers to Neospora antigens ranged from 40 to 160 before vaccination, from 80 to 5,120 2 weeks after the first dose of vaccination, and 320 to 40,960 2 weeks after the second (booster) vaccination. A peak antibody titer of 40,960 was also detected 28 days after the booster vaccination among animals vaccinated with Neospora vaccine formulated with Bay R1005 adjuvant. In heifers inoculated with experimental formulations of Neospora vaccines, transient development of injection site reactions resulted in 1 out of 15 animals. This injection site reaction was not detectable 14 days after the first observation and measurements were made. We have also demonstrated that vaccines derived from tissue- culture -grown **Neospora** tachyzoites are safe and would be expected to be efficacious.

Tags: Female

Descriptors: \*Antibodies, Protozoan--blood--BL; \*Cattle Diseases --prevention and control--PC; \*Coccidiosis--veterinary--VE; \*Neospora --immunology--IM; \*Protozoan Vaccines--immunology--IM; Animals; Cattle; Vaccination

CAS Registry No.: 0 (Antibodies, Protozoan); 0 (Protozoan Vaccines)

Record Date Created: 20010227

Record Date Completed: 20010301

?t s2/3,kwic/19 21 28 29 30 36 48 49 56 58 59 70 71 74 75

2/3,KWIC/19 (Item 1 from file: 5)

DIALOG(R)File 5:Biosis Previews(R)

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0014868771 BIOSIS NO.: 200400249528

**Recombinant neospora antigens and their uses**

AUTHOR: Conrad Patricia A (Reprint); Barr Bradd C; Anderson Mark L; Sverlow Karen W

AUTHOR ADDRESS: Davis, CA, USA\*\*USA

JOURNAL: Official Gazette of the United States Patent and Trademark Office  
Patents 1281 (1): Apr. 6, 2004 2004

MEDIUM: e-file

PATENT NUMBER: US 6716423 PATENT DATE GRANTED: April 06, 2004 20040406

PATENT CLASSIFICATION: 424-931 PATENT ASSIGNEE: The Regents of the  
University of California PATENT COUNTRY: USA

ISSN: 0098-1133 (ISSN print)

DOCUMENT TYPE: Patent

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: The present invention provides isolated bovine **Neospora cultures**. Also provided are recombinant immunodominant **Neospora** antigens. The **cultures** and antigens are used to develop diagnostic assays for the detection of Neospora infections in...

2/3,KWIC/21 (Item 3 from file: 5)

DIALOG(R)File 5:Biosis Previews(R)

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0014412576 BIOSIS NO.: 200300371295

**Adaptation in cell- culture of Neospora caninum isolates to changing environments: An argument in favor of its asexual propagation.**

AUTHOR: Perez-Zaballos F J (Reprint); Costas E (Reprint); Alvarez-Garcia G (Reprint); Collantes-Fernandez E (Reprint); Ortega-Mora L M (Reprint)

AUTHOR ADDRESS: Departamento Sanidad Animal, Facultad de Veterinaria,  
Universidad Complutense de Madrid, Ciudad Universitaria s/n, 28040,  
Madrid, Spain\*\*Spain

JOURNAL: Infection Genetics and Evolution 2 (4): p303 April 2003 2003

MEDIUM: print

CONFERENCE/MEETING: Sixth International Meeting on Molecular Epidemiology  
and Evolutionary Genetics of Infectious Diseases Paris, France July 23,

2002-July 27, 200320020723  
ISSN: 1567-1348 (ISSN print)  
DOCUMENT TYPE: Meeting; Meeting Abstract  
RECORD TYPE: Citation  
LANGUAGE: English

**Adaptation in cell- culture of Neospora caninum isolates to changing environments: An argument in favor of its asexual propagation.**

2/3,KWIC/28 (Item 2 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) Format only 2004 The Dialog Corp. All rts. reserv.

5597804 \*\*IMAGE Available  
Derwent Accession: 2000-664889

#### Utility

#### Recombinant neospora antigens and their uses

Inventor: Conrad, Patricia A., Davis, CA  
Barr, Bradd C., Davis, CA  
Anderson, Mark L., Davis, CA  
Sverlow, Karen W., Vacaville, CA  
Assignee: The Regents of the University of California (02), Oakland, CA  
Examiner: Navarro, Mark (Art Unit: 165)  
Law Firm: Townsend and Townsend and Crew L.L.P.

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 6716423	A	20040406	US 2000612858	20000710
Continuation	US 6376196	A		US 99281766	19990330
CIP	US 5889166	A	19990330	US 96645951	19960510
CIP	US 5707617	A	19980113	US 94327516	19941020
CIP	Abandoned			US 94215858	19940321

Fulltext Word Count: 19836

#### Abstract:

The present invention provides isolated bovine **Neospora cultures**. Also provided are recombinant immunodominant **Neospora** antigens. The **cultures** and antigens are used to develop diagnostic assays for the detection of **Neospora** infections in...

#### Description of the Invention:

...A "biologically pure bovine **Neospora culture**" refers to a continuous in vitro **culture** of bovine **Neospora** organisms (e.g. tachyzoites) which is substantially free of other organisms other than the host... **Neospora** tachyzoite **cultures** of the invention have been deposited with the American Type Culture Collection, Rockville, Md. on... antibodies raised against the tachyzoite of interest. By using these methods, immunodominant antigens from different **cultures** or species of **Neospora** can be identified...**Neospora** antigens. If isolated proteins are used, they may be recombinantly produced or isolated from **Neospora cultures**. Synthetic peptides made using the protein sequences may also be used...combinations of capture agent and labeled binding agent can be used. For instance, an isolated **Neospora** protein or **culture** can be used as the capture agent and labeled anti-bovine antibodies specific for the...The present invention provides biologically pure **cultures** of bovine **Neospora**. Two such **cultures** have been deposited with the ATCC and given ATCC Accession No. 75710 (BPA1), and ATCC...An **attenuated Neospora** vaccine can only be used in the absence of a risk of human infection should...Collection #CCL209) was found to be the best cell monolayer for the cultivation of bovine **Neospora**. One of these **cultures** (BPA6) has been shown to induce bradyzoite cysts in mice... these cows could be tested repeatedly over a 6 to 12 month period to determine **changes** in the **Neospora** titer. Peak titers of 640 to 2,560 were apparent within the first 20 daysThis example describes experimental

infections of pregnant cows with **culture** -derived **Neospora** tachyzoites ...In addition, polyclonal monospecific antisera to clones N54 and N57 only bound to **Neospora** antigens on **reducing** and nonreducing western blots. Rabbit anti-N54 recognized Neospora bands of molecular weights 97.2...

Non-exemplary or Dependent Claim(s):

- ...4. The method of claim 1, wherein the isolated bovine **Neospora** tachyzoite is **attenuated** .  
...9. The method of claim 6, wherein the isolated bovine **Neospora** tachyzoite is **attenuated** .  
...composition comprising a pharmaceutically acceptable carrier and an immunogenically effective amount of an isolated bovine **Neospora** tachyzoite that is **attenuated** , whereby the bovine animal is protected from abortion induced by Neospora infection...

2/3,KWIC/29 (Item 3 from file: 654)

DIALOG(R)File 654:US Pat.Full.

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0005594082

Derwent Accession: 1998-258985

Attenuated **live** neospora **vaccine**

Inventor: Brake, David, INV

Blagburn, Byron, INV

Lindsay, David, INV

Assignee: PFIZER INC (02), NEW YORK, NY

Correspondence Address: Dr. Peter C. Richardson Pfizer Inc., 235 East 42nd Street, New York, NY, 10017-5755, US

	Publication Number	Kind	Date	Application Number	Filing Date
	-----	--	-----	-----	-----
Main Patent	US 20040062772	A1	20040401	US 2003669941	20030924
Continuation	US 6656479			US 2001952388	20010912
Continuation	ABANDONED			US 99260414	19990226
Continuation	ABANDONED			US 97967744	19971110
Provisional				US 60-31248	19961112

Fulltext Word Count: 11700

Attenuated **live** neospora **vaccine**

Summary of the Invention:

...invention relates to attenuated strains of the pathogenic protozoan, Neospora, and to live vaccines against **neosporosis** prepared from the **attenuated** strains which are useful in the prevention of clinical disease and abortion in mammals...0005] WO 9525541 discloses a biologically pure **culture** of bovine **Neospora** , methods of detecting anti-Neospora antibodies and Neospora-specific nucleic acids, and a composition containing...

...Neospora antigen and carrier for use as a vaccine. WO 9525541 does not, however, teach **attenuated** live **cultures** of **Neospora** , or live vaccines prepared therefrom which are able to trigger a protective immune response in...of cells of a strain derived from a pathogenic parent strain of a species of **Neospora** , which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...

...live cells of a strain derived from a pathogenic parent strain of a species of **Neospora** , which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...live cells of a strain derived from a pathogenic parent strain of a species of **Neospora** , which cells exhibit **attenuated**

pathogenicity compared to those of the parent strain but which are capable of triggering an...

...live cells of a strain derived from a pathogenic parent strain of a species of **Neospora** , which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an

#### Description of the Invention:

...0109] The results indicate that a formulation comprising **attenuated** , live **Neospora** tachyzoites and an adjuvant is at least as effective and safe for use as a...0114] Table 20 demonstrates the ability of a vaccine comprising live, **attenuated** tachyzoites of **Neospora** to protect pygmy goat does against Neospora-induced abortion. All 4 goat does vaccinated with...

#### Exemplary or Independent Claim(s):

...of cells of a strain derived from a pathogenic parent strain of a species of **Neospora** , which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...

...live cells of a strain derived from a pathogenic parent strain of a species of **Neospora** , which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...live cells of a strain derived from a pathogenic parent strain of a species of **Neospora** , which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...live cells of a strain derived from a pathogenic parent strain of a species of **Neospora** , which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...

2/3,KWIC/30 (Item 4 from file: 654)

DIALOG(R)File 654:US Pat.Full.

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5451651

Derwent Accession: 1998-258985

#### Utility

C/ Attenuated **live** neospora vaccine  
; PROTECTS A MAMMAL **AGAINST NEOSPOROSIS**

Inventor: Brake, David A, East Lyme, CT  
Blagburn, Byron L, Auburn, AL  
Lindsay, David S, Christiansburg, VA

Assignee: Pfizer Inc. (02), New York, NY  
Pfizer Inc (Code: 65376)

Examiner: Smith, Lynette R. F. (Art Unit: 165)

Assistant Examiner: Portner, Ginny Allen

Law Firm: Scully, Scott, Murphy & Presser

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 6656479	A	20031202	US 2001952388	20010912
Continuation	Abandoned			US 99260414	19990226
Continuation	Abandoned			US 97967744	19971110

Fulltext Word Count: 9627

Attenuated **live** neospora vaccine...

#### Summary of the Invention:

...invention relates to attenuated strains of the pathogenic protozoan, Neospora, and to live vaccines against **neosporosis** prepared from the **attenuated** strains which are useful in the prevention of clinical

disease and abortion in mammals...WO 9525541 discloses a biologically pure **culture** of bovine **Neospora**, methods of detecting anti-**Neospora** antibodies and **Neospora**-specific nucleic acids, and a composition containing...

...**Neospora** antigen and carrier for use as a vaccine. WO 9525541 does not, however, teach **attenuated** live **cultures** of **Neospora**, or live vaccines prepared therefrom which are able to trigger a protective immune response in...

...of cells of a strain derived from a pathogenic parent strain of a species of **Neospora**, which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...live cells of a strain derived from a pathogenic parent strain of a species of **Neospora**, which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...live cells of a strain derived from a pathogenic parent strain of a species of **Neospora**, which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...live cells of a strain derived from a pathogenic parent strain of a species of **Neospora**, which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...

...Applicants have discovered that cells of a pathogenic strain of a species of **Neospora** may be **attenuated**, and that the resulting attenuated cells are capable of ...of cells of a strain derived from a pathogenic parent strain of a species of **Neospora**, which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...The term "attenuated" as used herein describes a cell, **culture**, or strain of **Neospora** exhibiting a detectable **reduction** in infectivity or virulence in vitro or in vivo as compared to that of the parent strain of **Neospora** from which the **attenuated** cell, culture, or strain is derived. Reduction in virulence encompasses any detectable decrease in any...Preparation of **Attenuated** Strains of **Neospora** ...Since the invention is based on the discovery that cells of a pathogenic strain of **Neospora** may be **attenuated**, and that the resulting attenuated cells are capable of triggering an immune response that protects...exposure, is preferably that amount which results in producing one or more viable cells of **Neospora** that exhibit an **attenuated** level of pathogenicity but that are capable of triggering an immune response that protects against...

...Pathogenic strains of **Neospora** may also be **attenuated** using recombinant DNA technology according to techniques known in the art, and the present inventionAn **attenuated** strain of **Neospora** may be derived from any pathogenic strain of any species of the genus including, but...

...Both parental strains and **attenuated** strains of **Neospora** may be **cultured** in vitro by infecting any receptive cell line, preferably a mammalian cell line, with tachyzoites...

...according to known techniques described in the art. Mammalian cell lines in which tachyzoites of **Neospora** can be **cultured** include, ... Mammalian cell cultures can be grown, and cell **cultures** infected with **Neospora** can be maintained, in any one of several culture media described in the art. For...

...but in which the fetal bovine serum is increased to 10% (v/v) (growth medium). **Attenuated** strains of **Neospora** having novel auxotrophies will require appropriate modification to the culture medium to support growth, as...

... **Neospora** -infected monolayer **cultures** of mammalian cells are typically maintained under standard tissue culture conditions such as, for example...

...live cells of a strain derived from a pathogenic parent strain of a species of **Neospora** , which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...

...live cells of a strain derived from a pathogenic parent strain of a species of **Neospora** , which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...The vaccine of the invention comprises live cells of an **attenuated** strain of **Neospora** , either as the sole antigenic component or in combination with one or more other antigens...

...live cells of a strain derived from a pathogenic parent strain of a species of **Neospora** , which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...formulations. Immunomodulatory agents are selected based on their ability to maintain both viability of the **attenuated Neospora** cells and ability of the cells to trigger a protective immune response in the vaccinated...term "protection" is used broadly and is not limited to absolute prevention of infection by **Neospora** , but includes a **reduction** in infectivity, or in the severity of a disease or condition resulting from infection, including...

Description of the Invention:

...The results indicate that a formulation comprising **attenuated** , live **Neospora** tachyzoites and an adjuvant is at ...Table 20 demonstrates the ability of a vaccine comprising live, **attenuated** tachyzoites of **Neospora** to protect pygmy goat does against Neospora-induced abortion. All 4 goat does vaccinated with...

2/3,KWIC/36 (Item 10 from file: 654)

DIALOG(R) File 654:US Pat.Full.

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0005091678

Derwent Accession: 1999-288171

**Neospora vaccines**

Inventor: Leszek Choromanski, INV

Karen Brown, INV

Correspondence Address: INTERVET INC, 405 STATE STREET PO BOX 318, MILLSBORO, DE, 19966, US

	Publication Number	Kind	Date	Application Number	Filing Date
	-----	--	-----	-----	-----
Main Patent	US 20020146436	A1	20021010	US 2002115478	20020402
Division	PENDING			US 97954531	19971020

Fulltext Word Count: 6469

Abstract:

A Neospora caninum vaccine comprising tissue **culture** grown **Neospora** and methods of making and using said vaccines. Neospora caninum vaccines described include those containing...

Summary of the Invention:

...this invention to describe a vaccine composition for protection of mammals from disease caused by **Neospora** caninum comprising tissue **culture** grown **Neospora** caninum tachyzoites as a whole culture or in an extract form or as subunit antigens...

...in a susceptible tissue culture until a cytopathic effect (CPE) is produced, harvesting said tissue **culture** grown **Neospora** caninum and formulating said harvest into a vaccine. A modified live vaccine produced in this...

...caninum in a susceptible tissue culture until a CPE is produced; 2) harvesting said tissue **culture** grown **Neospora** caninum; 3) inactivating said harvested tissue **culture** grown **Neospora** caninum; and 4) adjuvanting the inactivated harvested tissue **culture** grown **Neospora** caninum to produce a vaccine. Still another method of producing a vaccine for protection of...

...caninum in a susceptible tissue culture until a CPE is produced; 2) harvesting said tissue **culture** grown **Neospora** caninum; 3) extracting protective antigens from the harvested tissue **culture** grown **Neospora** caninum to produce subunits; 4) inactivating the subunits if necessary; and 5) adjuvanting the subunits...sup]6 TCID[sub]50/mL are produced. A Master Seed means that the tissue **culture** grown **Neospora** sp is grown to a high titer, aliquoted into equivalent volumes in freezing vials and ...mutated **Neospora** caninum in a susceptible tissue culture until CPE is produced, harvesting the tissue **culture** grown **Neospora** sp and formulating said harvest into a vaccine. Formulation may include addition of stabilizers and...

...**Neospora** caninum in a susceptible tissue culture until a CPE is produced, harvesting said tissue **culture** grown **Neospora** caninum, inactivating said harvested tissue **culture** grown **Neospora** caninum; and adjuvanting the inactivated harvested tissue **culture** grown **Neospora** caninum to produce a vaccine a susceptible tissue culture until a CPE is produced, harvesting said tissue **culture** grown **Neospora** caninum, extracting protective antigens from the harvested tissue **culture** grown **Neospora** caninum to produce protective antigen subunits, inactivating the subunits if necessary; and adjuvanting the subunits...

#### Description of the Invention:

...of DMEM. Each roller bottle contained 264 mL of DMEM plus 10% Horse Serum. The **neospora** -infected tissue **cultures** were incubated at 37 degree C. until at least 50% of the cells demonstrated CPE...

#### Exemplary or Independent Claim(s):

1. A **Neospora** caninum vaccine comprising tissue **culture** grown **Neospora** .

...

...in a susceptible tissue culture until a cytopathic effect is produced; b. harvesting said tissue **culture** grown **Neospora** caninum; and c. formulating said harvest into a vaccine...

...in a susceptible tissue culture until a cytopathic effect is produced; b. harvesting said tissue **culture** grown **Neospora** caninum; c. inactivating said harvested tissue **culture** grown **Neospora** caninum; and d. adjuvanting the inactivated harvested tissue **culture** grown **Neospora** caninum to produce a vaccine...

...in a susceptible tissue culture until a cytopathic effect is produced; b. harvesting said tissue **culture** grown **Neospora** caninum; c. extracting one or more protective antigens from the harvested tissue **culture** grown **Neospora** caninum to produce a subunit; d. inactivating the subunit(s), optionally; and e. adjuvanting the...

...in a susceptible tissue culture until a cytopathic effect is produced; b. harvesting said tissue **culture** grown **Neospora** caninum; c. inactivating the **Neospora** caninum harvest; d. extracting one or more protective antigens from the harvested, inactivated tissue **culture** grown **Neospora** caninum to produce a subunit(s); and e. adjuvanting the subunit(s) to produce a...

#### Non-exemplary or Dependent Claim(s):

2. The **Neospora** caninum vaccine according to claim 1 wherein the tissue **culture** grown **Neospora** comprises an antigen selected from the group consisting of a whole **culture** of **Neospora** tachyzoites, an inactivated tissue **culture** of **Neospora** tachyzoites, a modified

live tissue culture **Neospora** tachyzoites, an extract from  
Neospora tachyzoites and one or more subunits obtained from Neospora  
tachyzoites...

2/3,KWIC/48 (Item 1 from file: 349)  
DIALOG(R)File 349:PCT FULLTEXT  
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01106055

**LIVE ANTENUATED PARASITE VACCINE**  
**VACCIN DE PARASITE VIVANT ATTENUE**

Patent Applicant/Assignee:

AKZO NOBEL N V, Velperweg 76, NL-6824 BM Arnhem, NL, NL (Residence), NL  
(Nationality), (For all designated states except: US)

Patent Applicant/Inventor:

VAN POPPEL Nicole Francisca Johanna, Van Welderenstraat 105 A, NL-6511 MG  
Nijmegen, NL, NL (Residence), NL (Nationality), (Designated only for:  
US)

VERMEULEN Arnoldus Nicolaas, Korhoenderveld 34, NL-5431 HH Cuyk, NL, NL  
(Residence), NL (Nationality), (Designated only for: US)

SCHAAP Theodorus Cornelis, Van de Does de Willeboissingel 53, NL-5211  
CE's-Hertogenbosch, NL, NL (Residence), NL (Nationality), (Designated  
only for: US)

Legal Representative:

MESTROM J J L (et al) (agent), Intervet International B.V., P.O. Box 31,  
NL-5830 AA Boxmeer, NL,

Patent and Priority Information (Country, Number, Date):

Patent: WO 200426903 A2-A3 20040401 (WO 0426903)

Application: WO 2003EP10696 20030919 (PCT/WO EP03010696)

Priority Application: EP 200278953 20020920

Designated States:

(Protection type is "patent" unless otherwise stated - for applications  
prior to 2004)

AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ  
EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR  
LS LT LU LV MA MD MG MK MN MW MX MZ NI NO NZ OM PG PH PL PT RO RU SC SD  
SE SG SK SL SY TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW

(EP) AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LU MC NL PT RO SE  
SI SK TR

(OA) BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG

(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZM ZW

(EA) AM AZ BY KG KZ MD RU TJ TM

Publication Language: English

Filing Language: English

Fulltext Word Count: 12092

Fulltext Availability:

Claims

Claim

... 3, characterised in that said parasite belongs to the genus Eimeria,  
Cryptosporidium, Toxoplasma, Sarcocystis or **Neospora** . 5) **Attenuated**  
live parasite according to claim 2, characterised in that said parasite  
belongs to the family...

2/3,KWIC/49 (Item 2 from file: 349)  
DIALOG(R)File 349:PCT FULLTEXT  
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01008473 \*\*Image available\*\*

**SEROLOGICAL ASSAY FOR NEOSPOA CANINUM**  
**DOSAGE SEROLOGIQUE DE NEOSPOA CANINUM**

Patent Applicant/Assignee:

PFIZER PRODUCTS INC, Eastern Point Road, Groton, CT 06340, US, US  
(Residence), US (Nationality), (For all designated states except: US)



Patent Applicant/Inventor:

BRAKE David Alan, Pfizer Global Research and Development, Eastern Point Road, Groton, CT 06340, US, US (Residence), US (Nationality),  
(Designated only for: US)

RITTER Dianne Marie, Pfizer Global Research and Development, Eastern Point Road, Groton, CT 06340, US, US (Residence), US (Nationality),  
(Designated only for: US)

Legal Representative:

LUMB J Trevor (et al) (agent), c/o Simpson, Alison Urquhart-Dykes & Lord, 30 Welbeck Street, London W1G 8ER, GB,

Patent and Priority Information (Country, Number, Date):

Patent: WO 200338434 A2-A3 20030508 (WO 0338434)

Application: WO 2002IB4125 20021008 (PCT/WO IB0204125)

Priority Application: US 2001334811 20011031

Designated States:

(Protection type is "patent" unless otherwise stated - for applications prior to 2004)

AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ  
EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR  
LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI  
SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM ZW

(EP) AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LU MC NL PT SE SK TR

(OA) BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG

(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZM ZW

(EA) AM AZ BY KG KZ MD RU TJ TM

Publication Language: English

Filing Language: English

Fulltext Word Count: 2938

Fulltext Availability:

Detailed Description

Detailed Description

... with Neospora-based vaccines (see, e.g. U.S. Serial 5 No. 09/260,414 "**Attenuated Live Neospora Vaccine**", incorporated herein by reference and ...vaccinated on day 0 and day 21 with either a tachyzoite-based i) modified-live, **attenuated Neospora** vaccine (USPTO patent application serial 091260,414 "**Attenuated Live Neospora Vaccine**", incorporated herein by reference) or ii) inactivated, whole cell homogenate Neospora vaccine (USPTO patent...

2/3,KWIC/56 (Item 9 from file: 349)

DIALOG(R)File 349:PCT FULLTEXT

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00488951

**NEOSPORA VACCINES**

**VACCINS NEOSPORA**

Patent Applicant/Assignee:

BAYER CORPORATION,

Inventor(s):

CHOROMANSKI Leszek,

BROWN Karen K,

Patent and Priority Information (Country, Number, Date):

Patent: WO 9920303 A1 19990429

Application: WO 98US21515 19981013 (PCT/WO US9821515)

Priority Application: US 97954531 19971020

Designated States:

(Protection type is "patent" unless otherwise stated - for applications prior to 2004)

AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH  
GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW  
MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZW GH  
GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES  
FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN GW ML MR NE SN  
TD TG

Publication Language: English  
Fulltext Word Count: 6025

Fulltext Availability:  
Detailed Description  
Claims

#### English Abstract

A i(Neospora caninum) vaccine comprising tissue **culture** grown **Neospora** and methods of making and using said vaccines. i(Neospora caninum) vaccines described include those...

#### French Abstract

L'invention concerne un vaccin appele i(Neospora caninum) comprenant **Neospora** obtenue par **culture** tissulaire, et des procedes de production et d'utilisation desdits vaccins. Les vaccins i(Neospora...

#### Detailed Description

- ... this invention to describe a vaccine composition for protection of mammals from disease caused by **Neospora** caninum comprising tissue **culture** grown **Neospora** caninum tachyzoites as a whole culture or in an extract form or as subunit antigens...
- ...in a susceptible tissue culture until a cytopathic effect (CPE) is produced, harvesting said tissue **culture** grown **Neospora** caninum and formulating said harvest into a vaccine. A modified live vaccine produced in this...
- ...caninum in a susceptible tissue culture until a CPE is produced; 2) harvesting said tissue **culture** grown **Neospora** caninum; 3) inactivating said harvested tissue **culture** grown **Neospora** caninum; and 4) adjuvanting the inactivated harvested tissue **culture** grown **Neospora** caninum to produce a vaccine. Still another method of producing a vaccine for protection of...
- ...caninum in a susceptible tissue culture until a CPE is produced; 2) harvesting said tissue **culture** grown **Neospora** caninum; 3) extracting protective antigens from the harvested tissue **culture** grown **Neospora** caninum to produce subunits; 4) inactivating the subunits if necessary; and 5) adjuvanting the subunits...and more preferably, jX106 TCID50/mL are produced. A Master Seed means that the tissue **culture** grown **Neospora** sp is grown to a high titer, aliquoted into equivalent volumes in freezing vials and...
- ...mutated **Neospora** caninum in a susceptible tissue culture until CPE is produced, harvesting the tissue **culture** grown **Neospora** sp and formulating said harvest into a vaccine.

Formulation may include addition of stabilizers and...

- ...**Neospora** caninum in a susceptible tissue culture until a CPE is produced, harvesting said tissue **culture** grown **Neospora** caninum, inactivating said harvested tissue **culture** grown **Neospora** caninum; and adjuvanting the inactivated harvested tissue **culture** grown **Neospora** caninum ...**Neospora** caninum in a susceptible tissue culture until a CPE is produced, harvesting said tissue **culture** grown **Neospora** caninum, extracting protective antigens from the harvested tissue **culture** grown **Neospora** caninum to produce protective antigen subunits, inactivating the subunits if necessary; and adjuvanting the subunits...of DMEM. Each roller bottle contained 264 mL of DMEM plus 10% Horse Serum. The **neospora** -infected tissue **cultures** were

incubated at 37°C until at least 50% of the cells demonstrated CPE  
(approximately 7...

Claim

1. A *Neospora caninum* vaccine comprising tissue culture grown **Neospora**.

2 The *Neospora caninum* vaccine according to Claim 1 wherein the tissue culture grown **Neospora** comprises an antigen selected from the group consisting of a whole culture of **Neospora** tachyzoites, an inactivated tissue culture of **Neospora** tachyzoites, a modified live tissue culture **Neospora** tachyzoites, an extract from *Neospora* tachyzoites and one or more subunits obtained from *Neospora* tachyzoites...

...in a susceptible tissue culture until a cytopathic effect is produced;  
b. harvesting said tissue culture grown **Neospora caninum**; and  
c. formulating said harvest into a vaccine.

11 A method of producing a...

...in a susceptible tissue culture until a cytopathic effect is produced;  
b. harvesting said tissue culture grown **Neospora caninum**;  
c. inactivating said harvested tissue culture grown **Neospora caninum**; and  
d. adjuvanting the inactivated harvested tissue culture grown **Neospora caninum** to produce a vaccine.

12 A method of producing a *Neospora* subunit vaccine comprising...

...in a susceptible tissue culture until a cytopathic effect is produced;  
b. harvesting said tissue culture grown **Neospora caninum**;  
c. extracting one or more protective antigens from the harvested tissue culture grown **Neospora caninum** to produce a subunit;  
d. inactivating the subunit(s), optionally; and  
- 28  
e...

...in a susceptible tissue culture until a cytopathic effect is produced;  
b. harvesting said tissue culture grown **Neospora caninum**;  
c. inactivating the *Neospora caninum* harvest;  
d. extracting one or more protective antigens from the harvested, inactivated tissue culture grown **Neospora caninum** to produce

2/3, KWIC/58 (Item 11 from file: 349)  
DIALOG(R) File 349: PCT FULLTEXT  
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00376339

**A VACCINE AGAINST TOXOPLASMA GONDII**  
**VACCIN CONTRE TOXOPLASMA GONDII**

Patent Applicant/Assignee:

TRUSTEES OF DARTMOUTH COLLEGE,  
KASPER Lloyd H,  
KHAN Imtiaz A,  
MATSUURA Tadashi,

Inventor(s):

KASPER Lloyd H,

KHAN Imtiaz A,  
MATSUURA Tadashi,  
Patent and Priority Information (Country, Number, Date):  
Patent: WO 9717082 A1 19970515  
Application: WO 96US18100 19961108 (PCT/WO US9618100)  
Priority Application: US 956513 19951109  
Designated States:  
(Protection type is "patent" unless otherwise stated - for applications  
prior to 2004)  
AU CA JP US AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE  
Publication Language: English  
Fulltext Word Count: 2188  
  
Fulltext Availability:  
Claims

Claim

... Neospora antigen.

2 The vaccine of claim 1 wherein the Neospora antigen  
5 comprises live, **attenuated Neospora** .

3 The vaccine of claim 2 further comprising a  
pharmaceutically acceptable carrier.

4 The vaccine...

...The method of claim 6 wherein the Neospora antigen  
of the administered vaccine comprises live, **attenuated Neospora**

8 The method of claim 7 wherein the vaccine further  
comprises a pharmaceutically acceptable carrier...

2/3,KWIC/59 (Item 12 from file: 349)  
DIALOG(R)File 349:PCT FULLTEXT  
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00307389

**BOVINE NEOSPORA ISOLATES AND THEIR USES**  
**ISOLATS DE NEOSPORA BOVIN ET LEUR UTILISATION**

Patent Applicant/Assignee:

THE REGENTS OF THE UNIVERSITY OF CALIFORNIA,  
Inventor(s):

CONRAD Patricia A,  
BARR Bradd C,  
ANDERSON Mark L,  
SVERLOW Karen W,

Patent and Priority Information (Country, Number, Date):

Patent: WO 9525541 A1 19950928  
Application: WO 95US3174 19950314 (PCT/WO US9503174)  
Priority Application: US 94215858 19940321; US 94327516 19941020

Designated States:

(Protection type is "patent" unless otherwise stated - for applications  
prior to 2004)

AU CA JP NZ AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE

Publication Language: English

Fulltext Word Count: 15763

Fulltext Availability:

Detailed Description  
Claims

English Abstract

The present invention provides isolated bovine **Neospora cultures** . The  
**cultures** are used to develop diagnostic assays for the detection of  
Neospora infections in cattle and...

#### French Abstract

La presente invention concerne des **cultures** isolees de **Neospora** bovin. Ces **cultures** sont utilisees pour mettre au point des dosages diagnostiques en vue de la detection d...

#### Detailed Description

... blood, serum, plasma, urine,  
ascites fluid, cerebrospinal fluid and fetal fluid.

A "biologically pure bovine **Neospora culture** " refers to a continuous in vitro culture of bovine **Neospora** organisms (e.g. tachyzoites...from the cultures of the invention.

#### DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides **Neospora cultures** isolated from cattle. The cultures are useful in a variety of applications including the production...

...proteins for diagnostic assays and the preparation of immunogenic proteins for use in vaccine compositions.

**Neospora** tachyzoite **cultures** of the invention have been deposited with the American Type Culture Collection, Rockville, Maryland on...antigens. if 3S isolated proteins are used, they may be recombinantly produced or isolated from **Neospora cultures**. Synthetic peptides made using the protein sequences may also be used.

Methods of production of...combinations of capture agent and labelled binding agent can be used. For instance, an isolated **Neospora** protein or **culture** can be used as the capture agent and labelled anti-bovine antibodies specific for the...to either cattle or the definitive host can comprise tachyzoite and/or bradyzoite antigens.

An **attenuated Neospora** vaccine can only be used in the absence of a risk of human infection...CCL209) was found to be the best cell monolayer for the cultivation of bovine 35 **Neospora**. One of these **cultures** (BPA6) has been shown to induce bradyzoite cysts in mice.

#### MATERIALS AND METHODS

Pathological examination...these cows could be tested repeatedly over a 6 to 12 month period to determine **changes** in the **Neospora** titer.

Peak titers of 640 to 2,560 were apparent within the first 20 days...the Toxoplasma amplification product.

#### Example 5

This example describes experimental infections of pregnant cows with **culture**-derived **Neospora** tachyzoites.

Three cows were inoculated with  $8 \times 10^6$  tachyzoites of the BPA1 **Neospora** isolate...

#### Claim

1 A biologically pure **culture** of bovine **Neospora**.

2 The **culture** of claim 1, which is selected  
from the group consisting of ATCC Accession No. (BPA1...

...7 The method of claim 3, wherein the antigen  
is a protein isolated from a **Neospora culture** .

8 The method of claim 7, the isolated  
protein has a molecular weight of 106...

2/3,KWIC/70 (Item 10 from file: 348)  
DIALOG(R) File 348:EUROPEAN PATENTS  
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00923338

Attenuated **live neospora vaccine**  
Attenuierter **lebender Neospora Impfstoff**  
**Neospora vaccin vivant** atténue

PATENT ASSIGNEE:

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(US), (Proprietor designated states: all)  
AUBURN UNIVERSITY, (1311211), 309 Samford Hall, Auburn, AL 36849-5112,  
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INVENTOR:

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(US)  
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Lindsay, David, 1655 Sleepy Hollow Road, Christiansburg, Virginia 24073,  
(US)

LEGAL REPRESENTATIVE:

Green, Mark Charles (57752), Urquhart-Dykes & Lord LLP 30 Welbeck Street,  
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PATENT (CC, No, Kind, Date): EP 841392 A2 980513 (Basic)  
EP 841392 A3 990324  
EP 841392 B1 040714

APPLICATION (CC, No, Date): EP 97308133 971014;

PRIORITY (CC, No, Date): US 31248 P 961112

DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI; LU;  
NL; PT; SE

INTERNATIONAL PATENT CLASS: C12N-001/10; A61K-039/002; A61K-039/00;

C12N-001/10; C12R-1:90

ABSTRACT WORD COUNT: 34

LANGUAGE (Publication,Procedural,Application): English; English; English

FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	199820	527
CLAIMS B	(English)	200429	529
CLAIMS B	(German)	200429	522
CLAIMS B	(French)	200429	634
SPEC A	(English)	199820	8643
SPEC B	(English)	200429	8493
Total word count - document A			9173
Total word count - document B			10178
Total word count - documents A + B			19351

Attenuated **live neospora vaccine**  
Attenuierter **lebender Neospora Impfstoff**  
**Neospora vaccin vivant** atténue

...SPECIFICATION invention relates to attenuated strains of the pathogenic  
protozoan, Neospora, and to live vaccines against **neosporosis** prepared  
from the **attenuated** strains which are useful in the prevention of  
clinical disease and abortion in mammals.

BACKGROUND...

...al., 1995, Am. J. Vet. Res. 56:1176-1180.

WO 9525541 discloses a biologically pure **culture** of bovine **Neospora**, methods of detecting anti-Neospora antibodies and Neospora-specific nucleic acids, and a composition containing...

...Neospora antigen and carrier for use as a vaccine. WO 9525541 does not, however, teach **attenuated** live **cultures** of **Neospora**, or live vaccines prepared therefrom which are able to trigger a protective immune response in...

...of cells of a strain derived from a pathogenic parent strain of a species of **Neospora**, which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...

...live cells of a strain derived from a pathogenic parent strain of a species of **Neospora**, which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...

...live cells of a strain derived from a pathogenic parent strain of a species of **Neospora**, which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...

...live cells of a strain derived from a pathogenic parent strain of a species of **Neospora**, which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...

...THE INVENTION

Applicants have discovered that cells of a pathogenic strain of a species of **Neospora** may be **attenuated**, and that the resulting attenuated cells are capable of triggering an immune response that protects...

...of cells of a strain derived from a pathogenic parent strain of a species of **Neospora**, which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...

...to any clinical symptom, condition, event or pathology associated with infection of the mammal by **Neospora**.

The term "**attenuated**" as used herein describes a cell, **culture**, or strain of **Neospora** exhibiting a detectable **reduction** in infectivity or virulence in vitro or in vivo as compared to that of the parent strain of **Neospora** from which the **attenuated** cell, culture, or strain is derived. Reduction in virulence encompasses any detectable decrease in any...

...or antigen that triggers a protective immune response when administered to a mammal.

Preparation Of **Attenuated** Strains Of **Neospora**

Since the invention is based on the discovery that cells of a pathogenic strain of **Neospora** may be **attenuated**, and that the resulting attenuated cells are capable of triggering an immune response that protects...

...exposure, is preferably that amount which results in producing one or more viable cells of **Neospora** that exhibit an **attenuated** level of pathogenicity but that are capable of triggering an immune response that protects against...

...for use of mutagenic agents may be determined empirically using standard techniques.

Pathogenic strains of **Neospora** may also be **attenuated** using

recombinant DNA technology according to techniques known in the art, and the present invention...

...not replicate sufficiently and will thus fail to produce plaques in host cell monolayers.

An **attenuated** strain of **Neospora** may be derived from any pathogenic strain of any species of the genus including, but...

...monkey kidney cells from the ATCC (ATCC Accession No. CRL-12230).

Both parental strains and **attenuated** strains of **Neospora** may be **cultured** in vitro by infecting any receptive cell line, preferably a mammalian cell line, with tachyzoites...

...according to known techniques described in the art. Mammalian cell lines in which tachyzoites of **Neospora** can be **cultured** include, for example, human foreskin fibroblasts (Lindsay et al., 1993, Am. J. Vet. Res. 54...

...Bradyzoites may be similarly cultured and manipulated.

Mammalian cell cultures can be grown, and cell **cultures** infected with **Neospora** can be maintained, in any one of several culture media described in the art. For...

...but in which the fetal bovine serum is increased to 10% (v/v) (growth medium). **Attenuated** strains of **Neospora** having novel auxotrophies will require appropriate modification to the culture medium to support growth, as known in the art.

**Neospora** -infected monolayer **cultures** of mammalian cells are typically maintained under standard tissue culture conditions such as, for example...

...live cells of a strain derived from a pathogenic parent strain of a species of **Neospora**, which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...

...live cells of a strain derived from a pathogenic parent strain of a species of **Neospora**, which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...

...and a veterinarily acceptable carrier.

The vaccine of the invention comprises live cells of an **attenuated** strain of **Neospora**, either as the sole antigenic component or in combination with one or more other antigens...

...live cells of a strain derived from a pathogenic parent strain of a species of **Neospora**, which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...formulations. Immunomodulatory agents are selected based on their ability to maintain both viability of the **attenuated Neospora** cells and ability of the cells to trigger a protective immune response in the vaccinated...

...term "protection" is used broadly and is not limited to absolute prevention of infection by **Neospora**, but includes a **reduction** in infectivity, or in the severity of a disease or condition resulting from infection, including...

...of NCTS-8 does not produce clinical disease.

The results indicate that a formulation comprising **attenuated**, live **Neospora** tachyzoites and an adjuvant is at least as effective and safe for use as a...

...the attenuated NCTS-8 strain.

Table 20 demonstrates the ability of a vaccine comprising live, **attenuated** tachyzoites of **Neospora** to protect pygmy goat does against



Neospora-induced abortion. All 4 goat does vaccinated with...

...SPECIFICATION invention relates to attenuated strains of the pathogenic protozoan, *Neospora*, and to live vaccines against **neosporosis** prepared from the **attenuated** strains which are useful in the prevention of clinical disease and abortion in mammals.

BACKGROUND...

...al., 1995, Am. J. Vet. Res. 56:1176-1180.

WO 9525541 discloses a biologically pure **culture** of bovine **Neospora**, methods of detecting anti-*Neospora* antibodies and *Neospora*-specific nucleic acids, and a composition containing...

...*Neospora* antigen and carrier for use as a vaccine. WO 9525541 does not, however, teach **attenuated** live **cultures** of **Neospora**, or live vaccines prepared therefrom which are able to trigger a protective immune response in...

...of cells of a strain derived from a pathogenic parent strain of a species of **Neospora**, which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...

...live cells of a strain derived from a pathogenic parent strain of a species of **Neospora**, which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...

...live cells of a strain derived from a pathogenic parent strain of a species of **Neospora**, which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...

...live cells of a strain derived from a pathogenic parent strain of a species of **Neospora**, which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...

...THE INVENTION

Applicants have discovered that cells of a pathogenic strain of a species of **Neospora** may be **attenuated**, and that the resulting attenuated cells are capable of triggering an immune response that protects...

...of cells of a strain derived from a pathogenic parent strain of a species of **Neospora**, which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...

...to any clinical symptom, condition, event or pathology associated with infection of the mammal by **Neospora**.

The term "**attenuated**" as used herein describes a cell, **culture**, or strain of **Neospora** exhibiting a detectable **reduction** in infectivity or virulence in vitro or in vivo as compared to that of the parent strain of **Neospora** from which the **attenuated** cell, culture, or strain is derived. Reduction in virulence encompasses any detectable decrease in any...

...or antigen that triggers a protective immune response when administered to a mammal.

Preparation Of **Attenuated** Strains Of **Neospora**

Since the invention is based on the discovery that cells of a pathogenic strain of **Neospora** may be **attenuated**, and that the resulting attenuated cells are capable of triggering an immune response that protects...

...exposure, is preferably that amount which results in producing one or

more viable cells of **Neospora** that exhibit an **attenuated** level of pathogenicity but that are capable of triggering an immune response that protects against...

...for use of mutagenic agents may be determined empirically using standard techniques.

Pathogenic strains of **Neospora** may also be **attenuated** using recombinant DNA technology according to techniques known in the art, and the present invention...

...not replicate sufficiently and will thus fail to produce plaques in host cell monolayers.

An **attenuated** strain of **Neospora** may be derived from any pathogenic strain of any species of the genus including, but...

...monkey kidney cells from the ATCC (ATCC Accession No CRL-12230).

Both parental strains and **attenuated** strains of **Neospora** may be **cultured** in vitro by infecting any receptive cell line, preferably a mammalian cell line, with tachyzoites...

...according to known techniques described in the art. Mammalian cell lines in which tachyzoites of **Neospora** can be **cultured** include, for example, human foreskin fibroblasts (Lindsay et al., 1993, Am. J. Vet. Res. 54...

...Bradyzoites may be similarly cultured and manipulated.

Mammalian cell cultures can be grown, and cell **cultures** infected with **Neospora** can be maintained, in any one of several culture media described in the art. For...

...but in which the fetal bovine serum is increased to 10% (v/v) (growth medium). **Attenuated** strains of **Neospora** having novel auxotrophies will require appropriate modification to the culture medium to support growth, as known in the art.

**Neospora** -infected monolayer **cultures** of mammalian cells are typically maintained under standard tissue culture conditions such as, for example...

...live cells of a strain derived from a pathogenic parent strain of a species of **Neospora**, which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...

...live cells of a strain derived from a pathogenic parent strain of a species of **Neospora**, which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...

...and a veterinarily acceptable carrier.

The vaccine of the invention comprises live cells of an **attenuated** strain of **Neospora**, either as the sole antigenic component or in combination with one or more other antigens...

...live cells of a strain derived from a pathogenic parent strain of a species of **Neospora**, which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...formulations. Immunomodulatory agents are selected based on their ability to maintain both viability of the **attenuated Neospora** cells and ability of the cells to trigger a protective immune response in the vaccinated...

...term "protection" is used broadly and is not limited to absolute prevention of infection by **Neospora**, but includes a **reduction** in infectivity, or in the severity of a disease or condition resulting from infection, including...

...of NCT5-8 does not produce clinical disease.

The results indicate that a formulation comprising **attenuated**, live **Neospora** tachyzoites and an adjuvant is at least as effective and safe

for use as a...

...the attenuated NCTS-8 strain.

Table 20 demonstrates the ability of a vaccine comprising live, **attenuated** tachyzoites of **Neospora** to protect pygmy goat does against Neospora-induced abortion. All 4 goat does vaccinated with...

...CLAIMS of cells of a strain derived from a pathogenic parent strain of a species of **Neospora** , which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...

...live cells of a strain derived from a pathogenic parent strain of a species of **Neospora** , which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...

...live cells of a strain derived from a pathogenic parent strain of a species of **Neospora** , which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...

...CLAIMS of cells of a strain derived from a pathogenic parent strain of a species of **Neospora** , which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...

...live cells of a strain derived from a pathogenic parent strain of a species of **Neospora** , which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...

...live cells of a strain derived from a pathogenic parent strain of a species of **Neospora** , which cells exhibit **attenuated** pathogenicity compared to those of the parent strain but which are capable of triggering an...

...CLAIMS Neospora en vue de l'utilisation dans un vaccin qui protege un mammifere contre la **neosporose** , comprenant la **modification** des cellules provenant d'une souche parente pathogene d'une espece de Neospora; la selection...

...a 5.

12. Procede de preparation d'un vaccin pour proteger un mammifere contre la **neosporose** , comprenant la **modification** des cellules provenant d'une souche parente pathogene d'une espece de Neospora; la selection  
...

2/3,KWIC/71 (Item 1 from file: 357)

DIALOG(R)File 357:Derwent Biotech Res.

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0318775 DBR Accession No.: 2003-19915 PATENT

**New overexpressing homologous antigen vaccines, useful for immunization, prophylaxis or treatment of a vertebrate at risk of or suffering from, e.g. brucellosis, paratuberculosis, tuberculosis or neosporosis - vector expression in host cell for use in disease therapy and vaccine**

AUTHOR: SCHURIG G; BOYLE S M; SRIRANGANATHAN N

PATENT ASSIGNEE: SCHURIG G; BOYLE S M; SRIRANGANATHAN N 2003

PATENT NUMBER: US 20030044431 PATENT DATE: 20030306 WPI ACCESSION NO.:  
2003-521726 (200349)

PRIORITY APPLIC. NO.: US 268673 APPLIC. DATE: 20021011

NATIONAL APPLIC. NO.: US 268673 APPLIC. DATE: 20021011

LANGUAGE: English

...ABSTRACT: prophylaxis or treatment of a vertebrate at risk of or suffering from paratuberculosis, tuberculosis or **neosporosis** ,

comprises an **attenuated** or avirulent strain of a pathogenic bacteria of the genus *Brucella*, where the strain over...

2/3,KWIC/74 (Item 4 from file: 357)  
DIALOG(R)File 357:Derwent Biotech Res.  
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0238607 DBR Accession No.: 99-08708 PATENT  
**Vaccines containing *Neospora caninum* grown in tissue culture, useful for protecting cattle and dogs against infections and abortion - live attenuated subunit vaccine production**  
AUTHOR: Choromanski L; Brown K K  
CORPORATE SOURCE: Pittsburgh, PA, USA.  
PATENT ASSIGNEE: Bayer 1999  
PATENT NUMBER: WO 9920303 PATENT DATE: 990429 WPI ACCESSION NO.: 99-288171 (9924)  
PRIORITY APPLIC. NO.: US 954531 APPLIC. DATE: 971020  
NATIONAL APPLIC. NO.: WO 98US21515 APPLIC. DATE: 981013  
LANGUAGE: English

...ABSTRACT: *N. caninum*. (I) comprises an antigen selected from whole, inactivated or modified but live tissue **cultured *Neospora* spp.** tachyzoites or an extract or one or more subunits from these tachyzoites. (34pp)

DESCRIPTORS: ***Neospora caninum* tachyzoite live, attenuated**, subunit vaccine prep., tissue culture, appl. dog protection from abortion, infection mammal animal (Vol.18...

2/3,KWIC/75 (Item 5 from file: 357)  
DIALOG(R)File 357:Derwent Biotech Res.  
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**Live attenuated *Neospora* vaccine - *Neospora caninum* culture for vaccine production**  
AUTHOR: Brake D A; Blagburn B L  
CORPORATE SOURCE: New York, NY, USA; Auburn, AL, USA.  
PATENT ASSIGNEE: Pfizer; Univ.Auburn 1998  
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**Live attenuated *Neospora* vaccine - *Neospora caninum* culture for vaccine production**

DESCRIPTORS: ***Neospora caninum* culture**, appl. **attenuated *Neospora*** vaccine prep., dog, cow, goat, sheep, horse veterinary medicine protozoan mammal animal fermentation??? (Vol.17...

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**Phenotypic characterisation of a *Neospora caninum* temperature-sensitive strain in normal and immunodeficient mice.**

Dreier K J; Stewarter L W; Kerlin R L; Ritter D M; Brake D A

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International journal for parasitology (ENGLAND) Oct 1999, 29 (10) p1627-34, ISSN 0020-7519 Journal Code: 0314024

Document type: Journal Article

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The in vivo persistence, immunogenicity and pathogenicity of a recently described temperature-sensitive (ts) strain from *Neospora caninum*, NCTs-8, was investigated in normal and immunodeficient mice. Groups of BALB/c and SCID/Bg mice were infected s.c. with  $5 \times 10^6$  wild-type NC-1, control NCTs-8 (pass 0) or NCTs-8 tachyzoites prepared at four in vitro passage levels (pass 7, 13, 21 and 28). For persistence and immunogenicity studies, BALB/c mice were bled and sacrificed at 4, 6 or 8 weeks p.i. Sera were analysed by IFAT and brain tissues examined for lesions by histology and tested for parasite presence by PCR. For pathogenicity studies, SCID/Bg mice were monitored by clinical signs and survival time. Results from parasite persistence experiments demonstrated microscopic lesions and PCR positive brain tissues in NC-1 infected mice. In contrast, brain tissues from NCTs8-infected groups were consistently negative by histology and PCR. Based on IFAT titres, all parasite strains were immunogenic, although parasite-specific IgG levels were lower in the NCTs-8 infected groups. Results from pathogenicity studies in SCID/Bg mice demonstrated a significantly ( $P < 0.0001$ ) longer mean survival time in NCTs-8 vs NC-1 infected groups. In addition, there was no significant difference in mean survival time between control NCTs-8 and experimental passage NCTs-8 infected mice. Collectively, these studies demonstrate that the NCTs-8 strain maintains a stable phenotype following multiple passages in vitro, and possesses an **attenuated**, shorter persistence phenotype in vivo compared with the parental wild-type NC-1.

Descriptors: Coccidiosis--parasitology--PS; \* *Neospora* --physiology--PH; Animals; Antibodies, Protozoan--blood--BL; Brain--pathology--PA; DNA, Protozoan--analysis--AN; Mice; Mice, Inbred BALB C; Mice, SCID; *Neospora* --genetics--GE; *Neospora* --immunology--IM; *Neospora* --pathogenicity--PY; Phenotype; Polymerase Chain Reaction; Temperature; Virulence

CAS Registry No.: 0 (Antibodies, Protozoan); 0 (DNA, Protozoan)

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**Separation and cryopreservation of Neospora caninum tissue cysts from murine brain.**

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Journal of parasitology (UNITED STATES) Apr 1997, 83 (2) p319-21, ISSN 0022-3395 Journal Code: 7803124

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A protocol was developed for the separation, concentration enumeration, and cryopreservation of **Neospora** caninum tissue cysts from mouse brains. Brains from chronically infected mice were homogenized and tissue cysts counted in 10-microliters aliquots. Tissue cysts were separated from brain homogenates by centrifugation at 4,400 g on 35% (v/v) Percoll/phosphate-buffered saline (PBS) continuous-density gradients. After removal of the brain layer, the separated tissue cysts were concentrated by diluting the remaining solution with PBS and centrifuging at 500 g. The pellet was resuspended in PBS and tissue cysts were enumerated. Fifty percent of tissue cysts were recovered from brains centrifuged once and 64% from brains centrifuged twice. Tissue cysts were preserved with 7.5% dimethyl sulfoxide in horse serum at -60 C. After thawing, bradyzoites were digested in an acid/pepsin solution and placed onto Vero cell cultures.

**Neospora** caninum tachyzoites were recovered from cell cultures, indicating that bradyzoites retained viability after concentration and cryopreservation. Separated tissue cysts ranged in diameter from 107 microns to 15 microns (average = 31 microns), and the average bradyzoite dimensions were 2 x 7.5 microns. These methods make it possible to store viable N. caninum tissue cysts for oral- **infectivity** trials and other studies.

Tags: Support, U.S. Gov't, Non-P.H.S.

Descriptors: Brain--parasitology--PS; \*Cryopreservation; \* **Neospora** --isolation and purification--IP; Animals; Centrifugation, Density Gradient ; Cercopithecus aethiops; Mice; Vero Cells

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